DECLARATION UNDER 37 C.F.R §1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

1. I, Peter Feldman, Ph.D., am an inventor of the present application.

2. I have extensive experience in the fields of biochemistry and protein purification for many years. My Curriculum Vitae is attached herewith as Exhibit A.

3. I have read and understood Anderle et al. (U.S. Patent Application publication No. 2003/0133829). The primary teaching of the reference is of a gentle and effective procedure for inactivating pathogens in a protein solution, which does not substantially reduce the activity of a selected protein in the solution.

4. Anderle et al. teaches at paragraph [0005] that there is a serine protease group, which includes the prothrombin complex of coagulation factors, that are sensitive to and at least partially deactivated by the conventional S/D (solvent/detergent pathogen inactivation) method. The prothrombin complex of coagulation factors includes prothrombin and factor X which are the proteins of interest in our application. The reference recommends that, for these proteins, a detergent alone may be used at high concentrations.

5. Anderle et al. teaches at paragraph [0014] adding to a protein solution, either separately or in combination (a) from about 0.01 to about 2% by weight based on the total weight of the composition of a detergent; and (b) from about 0.001 to about 2% by weight based on the total weight of the composition of a carboxylic acid ester. The resulting mixture is then incubated for an amount of time sufficient to inactivate the pathogens. With this specific combination of a carboxylic acid ester and detergent it was surprisingly found that pathogens in a protein solution are effectively inactivated, while the protein activity is substantially fully preserved.

6. Anderle et al. teaches at paragraph [0086] that S/D treatments with solvents other than a carboxylic acid ester (e.g., with Tween (polysorbate) 80 and tri-n-butyl phosphate (TNBP)) reduce the activity of protein.

7. In view of the teachings of Anderle et al., a skilled person would have been discouraged from inactivating a solution containing prothrombin and/or Factor X with TNBP and detergent. Instead, the

teachings of Anderle et al. would have directed a skilled artisan to use a combination of a carboxylic acid ester and detergent.

8. The other inventors of the present invention and I were also concerned with preventing deactivation of prothrombin through use of the S/D technique. Despite the problems cited by Anderle et al. regarding this technique when using TNBP as solvent, we unexpectedly discovered that these problems could be avoided by first treating the prothrombin-containing fraction with TNBP and a detergent in the presence of Factor X. After this treatment, the entire fraction containing prothrombin, Factor X, TNBP and detergent is then loaded onto an anion exchange medium, followed by washing the anion exchange medium to remove TNBP and detergent. At that point, the Factor X can be activated by metal ions while still on the anion exchange medium to yield Factor Xa, which converts prothrombin to thrombin. In relation to Anderle's teachings that the S/D technique using TNBP as a solvent will deactivate prothrombin, the foregoing technique went against conventional wisdom and unexpectedly produced thrombin in high yields and at high specific activity.

9. We also discovered that high specific activity thrombin could be produced by a modification of the foregoing technique, wherein a solution containing Factor X is treated with TNBP and detergent, and then loaded onto an anion exchange medium. The anion exchange medium is washed to remove TNBP and detergent and the bound Factor X is subsequently activated to form Factor Xa by addition of metal ions. Next, virus-inactivated prothrombin is loaded onto the anion exchange medium, where the activated Factor Xa converts the prothrombin to thrombin.

10. We also discovered that high specific activity, virus-inactivated thrombin is generated when a solution containing Factor X and prothrombin are loaded onto an anion exchange medium followed by the steps of: subjecting the Factor X and prothrombin to a virus inactivation procedure by adding TNBP and detergent to the anion exchange medium, washing the anion exchange medium to remove TNBP and detergent, and activating Factor X on the anion exchange medium to form Factor Xa by addition of metal ions, wherein the Factor Xa then activates the prothrombin to yield thrombin. In relation to Anderle's teachings that the S/D technique using TNBP as a solvent will deactivate prothrombin, the foregoing technique also went against conventional wisdom and unexpectedly produced thrombin in high yields and at high specific activity.

11. We also discovered that high specific activity, virus-inactivated thrombin is generated when a solution containing Factor X is loaded onto an anion exchange medium followed by the steps of: subjecting the Factor X to a virus inactivation procedure by adding TNBP and detergent to the anion exchange medium, washing the anion exchange medium to remove TNBP and detergent, activating Factor X on the anion exchange medium to form Factor Xa by addition of metal ions, and loading virus-inactivated prothrombin onto the anion exchange medium, wherein the activated Factor Xa converts prothrombin to thrombin.

12. The importance of the specific order of steps in the methods outlined in the foregoing paragraphs is demonstrated in our specification in Example 9 on pages 25-27. Example 9 demonstrates that, after the S/D treatment, the thrombin generation must take place on the anion exchange medium. Sample A was a prothrombin complex concentrate which had not undergone any S/D inactivation step. In contrast, Samples B and C had been subjected to an S/D inactivation step. In Sample B, the S/D reagents were still present, whereas they had been removed from Sample C. Activation of all three samples was attempted by incubation with calcium ions in solution. No anion exchange medium was present during the activation of any of the samples. Despite the absence of any anion exchange medium, sample A generated significant amounts of thrombin. In contrast, in Samples B and C only negligible amounts of thrombin were recovered. These results indicate that the presence of the anion exchange medium is essential for the activation step when the prothrombin complex concentrate has previously been subjected to an S/D treatment step, regardless of whether or not the S/D reagents are removed prior to activation. In contrast, Examples 1-6, 10, 14 and 15 in our specification demonstrate that activation by addition of metal ions in the presence of the anion exchange medium leads to high levels of thrombin generation despite the previous S/D treatment step.

13. We have also found that removal of the S/D reagents from the anion exchange medium prior to the activation step resulted in significantly higher yields of thrombin. This is illustrated by the data below.

14. A solution containing prothrombin and factor X, which had previously been treated with polysorbate 80 detergent and tri-n-butyl phosphate (TNBP) solvent, was incubated at room temperature in the presence of DEAE-Sepharose CL6B anion-exchange gel and calcium ions (concentration: 2mmol/L or 40mmol/L). The solution either still contained the solvent-detergent reagents or had been depleted of solvent-detergent reagents by prior removal. Samples were taken at intervals and these were assayed for thrombin activity. The thrombin activity generated under the different conditions is shown in Table 1.

Table 1. Thrombin generation from S/D-treated prothrombin and factor X

	Thrombin activity, IU per mL of start material	
	With S/D reagents	S/D reagents removed
2mM calcium:		
Incubation 0-2 hours	Non-detectable	78
Incubation 28-30 hours	Non-detectable	397
40mM calcium:		
Incubation 0-2 hours	8.5	161
Incubation 28-30 hours	19.5	633

15. As can be seen from Table 1, at different calcium concentrations and incubation times, the presence of solvent and detergent reagents inhibited the generation of thrombin. Removal of the solvent and detergent reagents before the addition of calcium (metal ion) was necessary to achieve the efficient generation of thrombin. All the methods of the present invention include a washing step to remove the solvent and detergent prior to the activation step.

16. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 29th July 2010 By: P Feldman

Dr. Peter Feldman

Exhibit A
Peter Feldman Curriculum Vitae

Peter Anthony Feldman

Date of Birth	17th March 1956
Work Address	Bio Products Laboratory Dagger Lane Elstree Hertfordshire WD6 3BX, U.K.
Work Telephone	[+44] (0)20 8258 2326 (direct line)
Work e-mail address	peter.feldman@bpl.co.uk
Academic Qualifications	B.Sc. London University (Chemistry), 1977 Ph.D. Bristol University (Biochemistry), 1982

Education

Brighton, Hove & Sussex Grammar School	1967-1974
Chelsea College, University of London	1974-1977
University of Bristol	1977-1981

Employment

University of Bristol
- Research Assistant 1981-1983

Plasma Fractionation Laboratory
- Project Scientist 1983-1991
- Senior Project Scientist 1991-1992

Bio Products Laboratory
- Senior Project Scientist 1992-present
- Unit Manager, R&D 2003-present

Experience

Postgraduate CASE Award Industrial Training (1977)
Studied plasma fractionation methods at the Scottish National Blood Transfusion Service's Protein Fractionation Centre in Edinburgh.

Academic Research (1977-1983)
Developed purification methods for plasma and membrane-bound and investigated their forms of molecular assembly.
Ph.D. thesis, "Interactions between thrombin, antithrombin III and heparin"

Pharmaceutical Research and Development (1983-1992)
At the Plasma Fractionation Laboratory in Oxford:
- developed methods of virus-inactivation for coagulation factor concentrates;
- supported the manufacture of existing clinical biological products;
- developed and transferred new processes for the manufacture of pharmaceutical clotting factor concentrates;

Biopharmaceutical Project Management (1992 – present)
At BPL in Elstree, additional responsibilities included:
- technical strategy for therapeutic and non-clinical biological products and reagents;
- support and troubleshooting for prothrombin complex protein manufacture;
- project management;
- technology-transfer and re-engineering to/from third parties;
- negotiation and management of external research contracts;
- participation in international collaborative standardisation studies;
- preparation of pharmaceutical regulatory dossiers for UK, European and US agencies;

Strategic Management (1994 – present)
- development of corporate strategy proposals for biological products;
- liaison with the U.S. Food and Drug Administration, for international registration;
- reorganisation and change management;
- management of interdisciplinary projects;

Publications More than 30 publications in books, journals and meetings.

Membership Biochemical Society